It is clear from the specification that the inventors had possession of this invention, i.e. altering the sucrose storing capacity of a plant by transformation so as to regulate the PFP activity, at the time the application was filed.

There are many methods of transforming the plant so as to regulate the PFP activity, and the methods disclosed in the specification are only two examples. Once it was known that the sucrose content could be altered according to the invention, it would be obvious to a person skilled in the art that any of the known methods of transformation would be expected to work. It would not, however, have been obvious to a person skilled in the art, at the priority date, to regulate the sucrose content by altering the PFP activity.

Claim 3 has been amended to delete references to a variant of the nucleotide sequence and to a sequence which hybridizes to the sequence of Figure 1, in order to overcome the examiner's objections. The claim has further been amended to refer also to the sequence of Figure 2 (SEQ ID no:2), which is the full-length cDNA sequence of Figure 1 (SEQ ID No: 1).

The reference to an untranslatable form of the nucleotide sequences has not been deleted, as a person skilled in the art would easily know how to make the sequence untranslatable. One method, that of omitting the translation start codon, is disclosed in the specification at page 8 (paragraph 2) and Figure 3.

The applicants have also not deleted the reference to an antisense form of the nucleotide sequence, as this is simply a reversal of the nucleotide sequence, i.e. the 5' and 3' ends of the sequence will be reversed. The specification also teaches that an antisense form was used with success, at page 8 (paragraph 2) and Figure 4.

The applicants also refer to the Examiner's comments under point 11 (page 13 of the Office Action, last paragraph) "One would have had a reasonable expectation of success because both sense and antisense constructs are effective at inhibiting gene expression in a plant..."

It is apparent from the specification that the DNA of SEQ ID Nos: 1 and 2 is cDNA and not genomic DNA. The term "complementary" as used in claims 3, 6 and elsewhere in the specification does not refer to "cDNA" made from genomic DNA, but instead refers to the complement (i.e. the matching base) of the disclosed cDNA. In the case of *University of California v. Eli Lilly and Co,* the matter concerned the production of cDNA from genomic DNA of a particular organism.

Considerable work may be needed to produce or determine the sequence of the cDNA from genomic DNA (for example, in determining and removing the introns). However, in the present invention, the cDNA sequence has been disclosed, and the production of a complement of this cDNA is a routine task which does not involve determining what codons or nucleotide bases need to be removed. Furthermore, the sequences provided in the specification should serve as an adequate description for the complement thereof, as any skilled person would know what the complimentary bases should be. It is thus submitted that the present invention is clearly distinguishable from the *University of California* case.

Furthermore, the applicants also submit that it would be obvious to a person skilled in the art that fragments of the disclosed sequence could be used, as it is a well-known fact that fragments of genes which have as few as 21 nucleotides could be expected to have an effect on the activity of an enzyme. SEQ ID No:1 is also a fragment of SEQ ID No:2.

Applicants respectfully traverse the Examiner's rejection of claims 5, 15 and 16 under 35 U.S.C. §112, 1st paragraph.

The applicants are reluctant to deposit the vectors under the terms of the Budapest Treaty, as such a deposit is prohibitively expensive (South Africa does not have its own depositary authority and the South African currency is particularly weak). The applicants, however, are willing to provide an affidavit making assurances that the criteria set out for non-deposited biological material will be met (in particular that the vectors will be made available for the required number of years). A suitable affidavit is currently being prepared for signature, and will be submitted to the United States Patent Office as soon as the applicants have signed it before a notary public.

Applicants respectfully traverse the Examiner's rejection of claims 1-6, 8-14 and 17-30 under 35 U.S.C. §112, 1st paragraph.

The applicants have already stated that methods of down regulating or up regulating the enzyme activity would be obvious to a person skilled in the art, and has asserted that this is not the invention, but rather it is the fact that the sucrose content can be increased or decreased if the enzyme activity is down regulated or up regulated which is the inventive feature.

The Examiner's argument about the small changes to the sequences of the desaturases and hydroxylases is irrelevant. These changes lead to altered amino acid sequences, which change the activities of the enzymes. Homology dependent gene silencing is based on high levels (but not necessarily 100%) of homology on DNA (transcriptional) and RNA (post-transcriptional silencing) level. There are many cases where heterologous antisense or untranslatable sequences have led to the inhibition of specific genes.

The applicants also fail to see the relevance of the Examiner's "unpredictability of antisense inhibition" argument. Anybody skilled in the art will be able to relatively easily prepare a method or vector to down regulate gene expression. The details may vary, but will induce the same effect. Additionally, it is impossible to predict exactly how much inhibition will be obtained before a plant is transformed. Furthermore, if the PFP activity is down regulated, both its total and specific activities will decrease.

Applicants respectfully traverse the Examiner's rejection of claims 1-6, 8-15 and 17-30 under 35 U.S.C. §112, 2nd paragraph.

The Examiner's objections under point 7 have been addressed by the amendments.

Applicants respectfully traverse the Examiner's rejection of claims 1-4, 6, 8 and 12 under 35 U.S.C. §102(b).

The claims have been limited to sugarcane to address the Examiner's objections. The claims can be distinguished from Hajirezaei, because they now refer only to sugarcane plants. In addition, Hajirezaei does not disclose a method of increasing the sucrose content in tobacco. Instead, Hajirezaei deals with potato plants and not tobacco, and in both mature (Table 4) and sprouting (Table 7) tubers there was a reduction in sucrose concentrations or no significant changes following down regulation of the PFP enzyme. Additionally, Hajirezaei teaches an increase in sucrose cycling (i.e. synthesis and degradation), and not sucrose accumulation (end of abstract). Tables 10 and 11 referred to by Hajirezaei also support this teaching of increased sucrose cycling, and do not provide any evidence of increased sucrose accumulation.

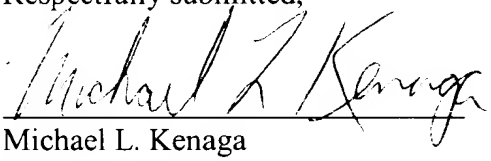
Applicants respectfully traverse the Examiner's rejection of claims 1-4, 6, 8-14 and 17-30 under 35 U.S.C. §103(a).

The applicants assert that it would not have been obvious at the time the invention was made to increase the sucrose content in sugarcane by down regulating the activity of the PFP enzyme. Hajirezaei does not teach that the sucrose content in potato tubers was increased by down regulation of the PFP enzyme, but instead teaches that the sucrose cycling (i.e. synthesis and degradation) was increased, with a reduction or no significant change in sucrose content. Neither Tanzer nor Chen teach that the sucrose content of a plant is increased, and therefore by combining Hajirezaei, Tanzer and Chen, it is submitted that there is no nexus, and a skilled person would not have thought it obvious, and would not have assumed a reasonable chance of success, that the sucrose content would be increased by the down regulation of the PFP enzyme.

Applicants have also attended to required changes to the drawings.

In view of the foregoing comments and amendment, applicants respectfully request reconsideration and to find the claims allowable over the prior art of record.

Respectfully submitted,



Michael L. Kenaga
Reg. No. 34,639

Piper Rudnick
P.O. Box 64807
Chicago, IL 60664-0807
Phone: (312) 368-4000
Fax: (312) 236-7516

Applicants: Botha, et al.
Serial No.: 09/779,237

EXHIBIT A

Marked-Up Version Of Amended Paragraphs Of The Specification Showing Changes Made

Please amend lines 8-26 of specification page 2 as follows:

In a preferred embodiment of the invention, the activity of the PFP enzyme is down regulated by the introduction of an untranslatable form or an antisense form of the nucleotide sequence as set out in Figure 1 (SEQ. ID No: 1), a nucleotide sequence which is complementary to the nucleotide sequence of Figure 1 (SEQ. ID No: 1), a variant of the nucleotide sequence of Figure 1 (SEQ. ID No: 1), a portion of the nucleotide sequence of Figure 1 (SEQ. ID No: 1), or a nucleotide sequence which hybridizes to the nucleotide sequence of Figure 1 (SEQ. ID No: 1), under stringent hybridization conditions.

The untranslatable or antisense nucleotide sequence may be introduced to the plant using plant expression vectors such as pUSPc 510 or pASPc 510.

According to the invention an isolated nucleotide sequence comprises:

(i) a nucleotide sequence as set out in Figure 1 (SEQ. ID No: 1);

(ii) a nucleotide sequence which is complementary to the nucleotide sequence of (i);

(iii) a variant of the nucleotide sequence of (i);

(iv) a portion of the nucleotide sequence of (i); or

(v) a nucleotide sequence which hybridizes to the nucleotide sequence of (i) under stringent hybridization conditions.

The nucleotide sequence may be the nucleotide sequence as set out in Figure 2 (SEQ. ID No: 2).

Please amend the last paragraph of specification page 4 as follows:

Figure 1 is the nucleotide sequence of the 1170 base pair (bp) cDNA fragment of clone PFP#5, containing the 3' end of the sugarcane PFP_{β} gene used in the construction of the plant expression vectors pUSPc 510 and pASPc 510 (SEQ. ID No: 1);

Please amend lines 1 and 2 of specification page 5 as follows:

Figure 2 is the complete cDNA nucleotide sequence of the sugarcane PFP_{β} gene (SEQ. ID No: 2);

Please amend the 2nd and 3rd paragraphs of specification page 6 as follows:

A first step of the invention was the cloning and characterization of a sugarcane PFP_{β} cDNA fragment. A set of degenerate primers was designed, based on the consensus of the castor bean and potato PFP_{β} gene sequences. These primers were used to amplify a fragment from sugarcane leafroll RNA which was then used as a probe to screen a sugarcane leafroll cDNA library for putative PFP_{β} clones. The sequence of the insert of one such clone is shown as an example in Figure 1 (SEQ. ID No: 1). This sequence contains a 1170 bp cDNA fragment. The complete sugarcane PFP_{β} coding sequence, as

shown in Figure 2 (SEQ. ID No: 2), was obtained by sequencing other cDNA and gDNA (genomic DNA) library clones.

The PFP_{β} cDNA fragment shown in Figure 1 (SEQ. ID No: 1) was excised and cloned into the plant expression vector pUBI 510 which confers high-level constitutive gene expression in sugarcane cells. One of the vectors, termed pUSPc 510, shown in Figure 3, contains a fragment in the sense orientation but it lacks a translation initiation codon, and is thus untranslatable. The other vector, termed pASPc 510, shown in Figure 4, contains a fragment in the antisense orientation.

Please amend lines 1 and 2 of specification page 8 as follows:

$_{\beta}$ coding sequence, as presented in Figure 2 (SEQ. ID No: 2), was obtained by sequencing other cDNA and gDNA library clones.

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EXHIBIT C

Marked-Up Version Of Amended Claims Showing Changes Made

1. (Amended) A method of regulating [and manipulating] sucrose content in a sugarcane [storing] plant [by] comprising up regulating or down regulating the activity of the PFP enzyme in the plant.

2. (Amended) A method according to claim 1 [wherein] which comprises increasing the sucrose content of the plant [is increased] by [the] down [regulation] regulating the activity of the PFP enzyme in the plant.

3. (Amended) A method according to claim 2 wherein the activity of the PFP enzyme is down regulated by [the introduction of] introducing an untranslatable form of an antisense form of the nucleotide sequence as set out in [Figure 1] either of SEQ. ID Nos: 1 or 2, or a nucleotide sequence which is complementary to the nucleotide sequence of SEQ. ID Nos: 1 or 2 [Figure 1, a variant of the nucleotide sequence of Figure 1, a portion of the nucleotide sequence of Figure 1, or a nucleotide sequence which hybridizes to the nucleotide sequence of Figure 1 under stringent hybridization conditions].

4. (Amended) A method according to claim 3 wherein the untranslatable or antisense nucleotide sequence is introduced into the plant using [a] plant expression vector, pUSPc 510 or pASPc 510.

6. (Amended) An isolated nucleotide sequence comprising:

(i) a nucleotide sequence as set out in either one of Figure 1 (SEQ. ID No: 1) or Figure 2 (SEQ. ID No: 2);

(ii) a nucleotide sequence which is complementary to the nucleotide sequence of (i); or

[(iii) a variant of the nucleotide sequence of (i);]

[(iv)](iii) a portion of the nucleotide sequence of (i)[; or] which is capable of up or down regulating the activity of the PFP enzyme in sugarcane.

[(v) a nucleotide sequence which hybridizes to the nucleotide sequence of (i) under stringent hybridization conditions.]

9. (Amended) A gene construct comprising a promoter and a nucleotide sequence as defined in claim 6 in [a] the sense orientation, the gene construct lacking a translation initiation codon upstream of the nucleotide sequence or possessing an in-frame termination codon directly downstream of the initiating codon.

15. (Amended) The plant expression vector pUSPc 510 which includes the nucleotide sequence of Figure 1 (SEQ. ID No: 1) or Figure 2 (SEQ. ID No: 2) in a sense orientation, but in an untranslatable form.

16. (Amended) The plant expression vector pASPc 510 which includes the nucleotide sequence of Figure 1 (SEQ. ID No: 1) or Figure 2 (SEQ. ID No: 2) in an antisense orientation.

17. (Amended) A transformed sugarcane plant cell which includes a gene construct according to claim 9.

18. (Amended) A transgenic plant or plant part containing [or derived from] the transformed plant cell of claim 17.

20. (Amended) A transformed plant cell according to claim 17 which is characterized by a lower level of the PFP_{β} protein, relative to an untransformed plant.

21. (Amended) A transformed plant or plant part according to claim 18 characterized by a lower level of the PFP_{β} protein, relative to an untransformed plant.

22. (Amended) A transformed plant cell according to claim 17 characterized by a lower level of PFP activity, relative to an untransformed plant.

23. (Amended) A transgenic plant or plant part according to claim 18 characterized by a lower level of PFP activity, relative to an untransformed plant.

24. (Amended) A transformed plant call according to claim 17 characterized by a higher level of sucrose, relative to an untransformed plant.

25. (Amended) A transgenic plant or plant part according to claim 18 characterized by a higher level of sucrose, relative to an untransformed plant.

26. (Amended) A method of regulating [or manipulating] the level of active PFP in a sugarcane plant cell [including] comprising the step of transforming the plant cell with at least one gene construct according to claim 9.

27. (Amended) A method of [maintaining or] increasing the sucrose level in sugarcane plant tissue [including] comprising the step of transforming cells of the plant tissue with at least one gene construct according to claim 9.

28. (Amended) A method of [manipulating] increasing sucrose metabolism in a plant cell of a sugar-storing plant [including] comprising the step of co-transforming the cell with a gene construct according to claim 9.